

MONITORING THE QUALITY OF ESSENTIAL OIL FROM *ETLINGERA* SP.4
(ZINGIBERACEAE) BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY
(GC-MS)

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A report submitted in partial fulfillment of the requirements for the award of the
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I declare that this thesis entitled “*Monitoring the Quality of Essential Oil from Etlingera sp.4 (Zingiberaceae) by Gas Chromatography-Mass Spectrometry (GC-MS)*” is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

Signature :

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Date :

*Special dedication to my family members, my supervisor, my beloved friends and all
faculty members*

For all your love, care support, and believe in me. Thank you so much.

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ABSTRACT

Essential oils are volatile, fragrant oils that occur in plants and in general contribute to their characteristic odors, flavors, or other properties. Studies have revealed that there is a spectrum of essential oils present in Zingibereaceae species, which are used widely as spice, flavouring and medicinal sources. The focus of current research was given to a wild species named as sp.4 from the genus *Etlingera* whose most species are still in undeveloped stage. The extraction of essential oil from the rhizome material of *Etlingera* sp.4 was conducted by hydro-distillation process using an all glass Clevenger-type apparatus. The compositions of essential oil isolated were further analyzed by gas chromatography-mass spectrometry (GC-MS) analysis. In addition, the effects of storage conditions including exposure to heat and light on the quality of essential oil were observed for 3 weeks. The changes in the compositions of essential oil were monitored by comparing the area of peaks. Based on the quantitative analysis of the GC-MS results, it was discovered that extreme storage conditions will result in the loss of certain volatile oil components in essential oils. Thus, optimum storage condition must be applied on natural essential oil to preserve its freshness and potency.

ABSTRAK

Minyak pati merupakan minyak yang mudah meruap dan berbau wangi yang menyumbangkan bau, rasa dan ciri-ciri penting lain kepada tumbuhan yang berkenaan. Kajian telah mendapati bahawa terdapat pelbagai jenis minyak pati yang wujud dalam spesies keluarga Zingiberaceae. Minyak pati halia digunakan secara meluas sebagai rempah, bahan perasa dan sumber perubatan. Kajian ini memberi tumpuan terhadap sejenis spesies dari genus *Etlingera* yang dinamakan sebagai sp.4. Minyak pati daripada bahagian rizom *Etlingera* sp.4 akan diekstrak menggunakan kaedah penyulingan hidro lalu dianalisa menggunakan GC-MS. Kesan pendedahan cahaya dan haba terhadap kualiti minyak pati tersebut juga diperhatikan selama 3 minggu. Perubahan komposisi minyak pati diperhatikan dengan membandingkan keputusan GC-MS. Berdasarkan analisis GC-MS secara kuantitatif, didapati bahawa kaedah penyimpanan yang kurang sesuai akan menyebabkan kehilangan sebahagian kandungan minyak pati dan mengurangkan kualitinya. Oleh itu, minyak pati semula jadi wajarlah disimpan dalam keadaan yang optimum untuk mengekalkan kesegaran dan kualitinya.

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Herbs have been extensively used throughout human history as sources of food, medicine, beauty enhancers, and fragrances. The herbal-related market includes herbs used as food or food additives, cosmetic ingredients, and herbal medicines. The current estimates for this market ranges between USD 40 to 100 billion with an average growth rate of 15 to 20 percent annually. (Ramlan, 2006)

In view of the potential size of market, it is important for Malaysia to build up herbal industry with its rich biological heritage, cultural background, and trade links. In fact, Malaysia is listed as the 12th most bio-diverse nation in the world and ranks 4th in Asia with over 15,000 flowering plants and over 3000 species medicinal plants. However, only about 50 out of the 3000 species are used commercially and even less are researched scientifically for their medicinal properties.

Among the valuable species of medicinal plants, local scientists and researchers have shown increasing interests in plants of Ginger family due to its wide potentials. Studies have revealed that there is a spectrum of essential oils present in Zingiberaceae species (Ibrahim & Zakaria, 1987; Zakaria *et al.*, 1989). A large number of species, particularly the strongly aromatic ones, could be screened to production of medicinal essential oils. Many active components, such as Zingiberene, have been found in the essential oil of ginger.

Ginger (*Zingiber officinale*) has a long history of being used as a spice and medicinal source. Chinese use ginger as common traditional medicine for the treatment of many diseases, such as common cold (Yu *et al.*, 2006). In recent years, more and more pharmaceutical effects have been found about ginger. It can act as an aphrodisiac, a carminative, a rubifacient, an anti-asthmatic and as a stimulant to the gastrointestinal tract (U. Bhandari, 1998). Ginger is often used for the treatment of stomachache, and cardiovascular and motor diseases. It also possesses anti-inflammatory activity and regulates bacterial growth, as well as providing protection for immune-depressed patients who are HIV positive (Penna, 2003).

The family of Zingiberaceae is conventionally classified into distinct genera, each genus consists of usually several species. Some examples of distinct genera are *Curcuma*, *Kaempferia*, *Alpinia*, *Zingiber* and *Etlingera*. The focus of current research will be given to the genus *Etlingera* whose most species are still in undeveloped stage. Until now there are still no comprehensive reports on the use of rhizomes of *Etlingera* species. As a matter of fact, all ginger plants of genus *Etlingera* contain essential oil, where the highest content usually exists in rhizomes part.

Since *Etlingera elatior* or “kantan” has been extensively studied, the present study will thus concentrate on a wild species of *Etlingera* collected near Bentong. While comprehensive morphological examination is being carried out by the botanist at Universiti Malaya, the specimen will be named *Etlingera* sp. 4. The properties of essential oil extracted from *Etlingera* sp. 4 will be investigated in detail.

In general, an analytical procedure for essential oil from natural plants comprises two steps which are extraction and analysis. The Clevenger-type apparatus based on hydro-distillation will be used to extract essential oil from specimen in this case. Hydro-distillation is recommended for small scale operation as it protects the quality of essential oil from heat destruction. On the other hand, gas chromatography-mass spectrometry (GC-MS) is applied to determine the composition of essential oil for their reliable accuracy.

1.2 Objective

The main objective of the present study is to monitor the quality of essential oil from *Etlingera* sp.4 (Zingiberaceae) by gas chromatography-mass spectrometry (GC-MS).

1.3 Scope of Study

In order to achieve the objectives of this research, the scopes need to be identified. At initial stage, the study involves collecting rhizome samples of *Etlingera* sp.4 (Zingiberaceae) from a nearby forest and preparation of samples before the extraction process. The pre-treatment process of samples is immediately followed by the isolation of essential oils by hydro-distillation process using an all glass Clevenger-type apparatus.

After purification procedure, the profile of pure essential oil will be further monitored by gas chromatography-mass spectrometry (GC-MS) analysis. A most suitable analysis method will be developed by trial and errors.

In addition, the effects of storage conditions including exposure to heat and light on the quality of essential oil will be observed. The storage condition is important to preserve the quality and freshness of natural essential oil.

1.4 Problem Statement

GC/MS analysis of the essential oil from the rhizome material of *Etlingera* sp. 4 has never been reported before. Research work is essential to develop the potentials from rhizome part of genus *Etlingera*.

In addition, the quality of natural essential oils is easily affected by its storage

conditions such as exposure to light and air as well as the changes in surroundings temperature. Appropriate handling and processing methods are crucial to minimize the loss of volatile oil components in essential oil.

Therefore, it is desirable to develop potential methods where the qualities of essential oil can be monitored efficiently. In the present study, the properties of essential oils under various conditions are interpreted from the result of gas chromatography-mass spectrometry (GC-MS) analysis. The results yield useful information on the effects of storage conditions to quality of essential oil.

CHAPTER 2

LITERATURE REVIEW

2.1 Ginger

2.1.1 Overview of Ginger

The word “Ginger” comes from the ancient Sanskrit word “singabera”, meaning shaped like a horn. Ginger is a tropical herb extensively grown for its pungently aromatic underground stem or rhizome which is an important export crop valued for its powder, oil and oleoresin (NEPC, 1999). It is a very valuable herbaceous perennial, said to be originated from central Asia and was among the first vegetatively cultivated plants. Today, ginger is cultivated all over tropic and sub-tropic Asia, where 50% of the world's harvest is produced in India. On the other hand, approximately 1000 species occur in tropical Asia while about 600 species are present in South East Asia.

The Zingiberaceae, or Ginger family, is a moderately-sized family of relatively advanced monocotyledonous plants of the order Zingiberales. The family is conventionally classified into at least 51 genera and 1500 species (Chen & Chen, 1998). Some distinct genera include *Curcuma*, *Kaempferia*, *Alpinia*, *Zingiber* and *Etlingera*.

Zingiberaceous species provide potential resources for a variety of uses ranging from medicine to food. The plants are basically rhizomatous, aromatic herbs

ranging in size from as small as 15cm in *Camptandra parvula* to as tall as 5m as observed in a number of genera from the tribe *Alpineae*. A comprehensive introduction to Zingiberaceae can be found in earlier reports and papers (Ibrahim, 1989; 1990).

2.1.2 Physical Properties

Zingiberaceous plants are rhizomatous, perennial and aromatic herbs often of large size, bearing white or yellowish-green flowers either terminally on aerial leaf shoots or from ground level.

Ginger is a knotted, thick, beige underground stem (rhizome). The stem extends roughly from one to three feet above ground, surrounded by the long, narrow, spear-shaped and green leaves. The rhizome produces a refreshing and lemon-like smell, as well as pungent taste.

2.1.3 Chemical Composition

Ginger contains ginger oil and oleoresin that account for the characteristic aroma of ginger. On the other hand, the oleoresin fraction of ginger rhizomes consists of both volatile oils and nonvolatile pungent compounds which can be extracted with solvents such as acetone or alcohol.

The volatile oil components in ginger consist mainly of sesquiterpene hydrocarbons, predominantly zingiberene (35%), curcumene (18%) and farnesene (10%). An insignificant percentage of at least 40 different monoterpene hydrocarbons are present with 1, 8-cineole, linalool, borneol, neral, and geraniol being the most abundant (Combest, 2003). Many of these volatile oil constituents contribute to the distinctive aroma and taste of ginger, however most of them are not unique to ginger.

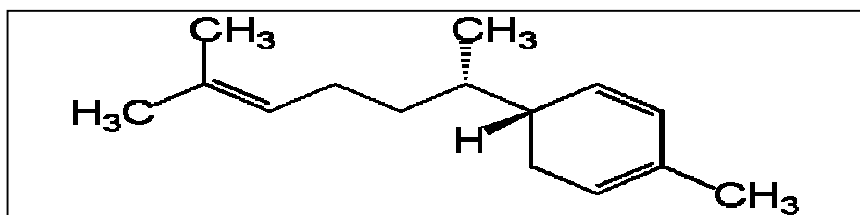


Figure 2.1 Structure of Zingiberene (A. Heinrich, 2004)

Several nonvolatile constituents of ginger are being responsible for its characteristic pungent flavor as well as pharmacological actions. The principle components of this fraction are the gingerols and shogaol. Shogaol is dehydroxylated derivatives of gingerols whose concentrations increase in dried ginger after prolonged storage.

In addition to the extractable oleoresins, ginger contains many fats, waxes, carbohydrates, vitamins and minerals. Ginger rhizomes also contain a potent proteolytic enzyme called zingibain.

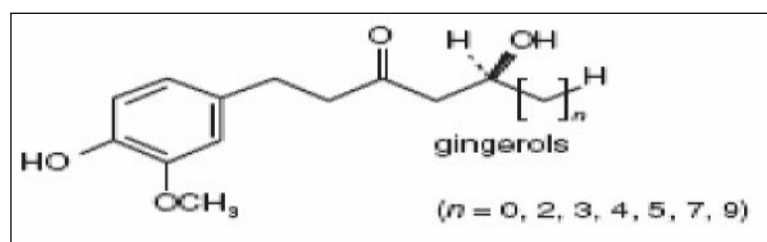


Figure 2.2 Structure of Gingerol (A. Heinrich, 2004)

2.2 Genus *Etlingera*

2.2.1 Overview

The genus *Etlingera* from the family of Zingiberaceae is distributed from India to the Pacific Islands with centres of species richness are assumed in Borneo and New Guinea. Presently at least 70 species are known from the Malesian region, which refers to a floristically distinct region including Malaysia, Indonesia, Brunei, Singapore, Phillipines and Papua New Guinea (Poulson, 2002). Works by Lim (2001) shown that a total of 15 *Etlingera* species has been recorded in Peninsular Malaysia.

2.2.2 Physical Properties

Species of *Etlingera* can be more than 5 m tall and become dominant in gaps. *Etlingera Giseke* of the family Zingiberaceae are tall forest plants, with larger species reaching 6 m in height (Khaw, 2001). In the *Phaeomeria* group, inflorescences are borne on erect stalks protruding from the ground; whereas in the *Achasma* group, inflorescences are subterranean with flowers appearing at soil level (Lim, 2000, 2001). The varying shades of pink and red colours of bracts and flowers make *Etlingera* species well known for their attractiveness.

2.2.3 Usage

Etlingera is among the most diverse and attractive genera found in Ginger family. Plants of *Etlingera* display high commercial values for their wide applications and usages. In Sabah, Malaysia, the hearts of young shoots, flower buds and fruits of *E. elatior*, *E. rubrolutea*, and *E. littoralis* are consumed by indigenous communities as condiment, either eaten raw or cooked (Noweg, Abdullah, & Nidang, 2003). In

Thailand, fruits and cores of young stems of *E. littoralis* and flowers of *E. maingayi* are eaten as vegetables (Sirirugsa, 1999).

Inflorescences of *E. elatior* or locally known as “bunga kantan” are widely cultivated throughout the tropics as spices for food flavouring and as ornamentals. They are commonly used as ingredients of some local dishes such as *laksa asam*, nasi kerabu, and nasi ulam in Peninsular Malaysia (Larsen *et al.*, 1999). In addition, farms in Australia and Costa Rica are cultivating the species and selling its inflorescences as cut flowers (Larsen *et al.*, 1999).

For medicinal uses, fruits of *E. elatior* are used traditionally to treat earache, while leaves are applied for cleaning wounds in Malaysia (Ibrahim & Setyowati, 1999). Leaves of *E. elatior*, mixed with other aromatic herbs in water, are used by post-partum women for bathing to remove body odour.



Figure 2.3 *Etlingera elatior* (P. Ròebère, 2003)

2.3 Essential Oils

Essential oils are volatile, fragrant oils that occur in plants and in general contribute to their characteristic odors, flavors, or other such properties (Heravi, 2006). They are found in various parts of the plant body such as seeds, flower petals, bark, rhizomes, roots or leaves. They are also concentrated in certain special groups of cells.

Essential oils are extremely concentrated form of any botanical. For instance, essential oils made from rose plants require 4,000 pounds of rose petals to make one pound of essential oil. This explains the high commercial value of essential oils.

Essential oils have been used for thousands of years. Essential oils were the primary source of perfumes for the ancient civilizations of Egypt, India, Greece, and Rome. Nowadays, owe to their special properties, essential oils are widely used as perfumes, food flavorings, medicines and as fragrant and antiseptic additives in many common products. Essential oils are also applied in the healing practice of aromatherapy, one of the fastest-growing alternative health treatments in the 1990s.

Natural essential oils can be isolated from plants by various techniques, depending upon the nature of the plant body. Distillation process is widely applied where water or steam is used to remove the essential oils from dried or fresh plants. Mechanical expression method is sometimes useful to squeeze the oil out of plants by machines. Furthermore, other techniques may use alcohol or solvents to extract essential oils from plant materials with delicate chemical components.

Unlike vegetable oils expressed from nuts and seeds, essential oils are not oily. Essential oils are not fat-based, but they have a lipid-soluble molecular structure which allows them to pass easily to human skin. Essential oils are highly volatile, they evaporate into air easily. In addition, essential oils are sensitive to heat and light. Thus, they should be stored in dark or wrapped bottles and places with appropriate temperature to preserve their quality.

2.4 Separation Processes

Separation Processes is defined as any set of operations that separate two or more components into two or more products that differ in composition (Noble & Terry, 2004). The aim of separation is attained by exploiting the differences between chemical and physical properties of the substances through mass or energy.

Most chemical materials and biological substances occur in mixture form of different components in the gas, liquid, or solid phase. To separate one or more of components from the original chemical mixture, it must be contacted with another phase. The two phases are brought into more or less intimate contact with each other so that a solute or solutes can diffuse from one to the other. (Geankoplis, 2003)

The two bulk phases are usually miscible in each other to certain extent only. The two-phase pair can be gas-liquid, gas-solid, liquid-liquid, or liquid-solid. During the contact of the two phases, the components of the original mixture redistribute themselves between the two phases. The phases are then separated by simple physical methods. By choosing the proper conditions and phases, one phase is enriched while the other is depleted in one or more components.

There are a few examples of common separation process:

i. Absorption

When the two contacting phases are a gas and liquid, this operation is called absorption. A solute or several solutes are absorbed from the gas into the liquid phase in absorption.

ii. Distillation

In the distillation process, a volatile vapor phase and a liquid phase that vaporizes are involved.

iii. Liquid-liquid extraction

When the two phases are liquids, where a solute or solutes are removed from one liquid phase to another liquid phase, the process is called liquid-liquid extraction.

iv. Leaching

If a fluid is being used to extract a solute from a solid, the process is called leaching. Sometimes this process is also called extraction.

v. Membrane processing

Separation of molecules by the use of membranes is a relatively new separation process and is becoming more important. The relatively thin, solid membrane controls the rate of movement of molecules between two phases.

vi. Crystallization

Solute components soluble in a solution can be removed from a solution by adjusting the conditions, such as temperature or concentration, so that the solubility of one or more of the components is exceeded and they crystallize out a solid phase.

vii. Adsorption

In an adsorption process, one or more components of a liquid or gas stream are adsorbed on the surface or in the pores of a solid adsorbent and a separation is obtained.

viii. Ion exchange

In an ion exchange process, certain ions are removed by an ion-exchange solid. This separation process closely resembles adsorption.